Correlation Between SRID and ELISA of Serum Immunoglobulin G Concentrations in Quarter Horse Mares and Their Foals From Birth to Weaning

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ABSTRACT

Failure of passive transfer (FPT) is a life threatening condition in foals and occurs when immunoglobulin G concentrations (IgG) are below 400 mg/dL and partial failure of passive transfer (PFPT) occurs when IgG concentrations are between 400-800 mg/dL. An IgG concentration of greater than 800 mg/dL is considered sufficient to protect the foal against pathogens. The standard method of determining antibody concentrations is Single Radial Immunodiffusion (SRID). However, this method requires an 18 h incubation and is not time efficient. Enzyme Linked Immunosorbant Assays (ELISA) only require a 4 h incubation period to detect IgG concentrations. The purpose of this study is the correlate the immunoglobulin G concentrations of mares and foals derived from ELISA with a previous study conducted using SRID. A low correlation between ELISA and SRID was found and within blocks, foals displayed the highest correlations.

INTRODUCTION

Antibodies, also known as immunoglobulins, are proteins secreted by B cells in response to an antigen present in the body (Mayer, n.d.). Antibodies are antigen specific and function by binding to the antigen to neutralize it. There are five main classes of antibodies: immunoglobulin G (IgG); immunoglobulin M (IgM); immunoglobulin A (IgA); immunoglobulin E (IgE); and immunoglobulin D (IgD). Each immunoglobulin performs a different function. Immunoglobulin G constitutes about 75% of serum immunoglobulin and considered the most

versatile immunoglobulin because it can perform several functions including antigen binding, fixation of complement, and binding to various cells like phagocytic cells, lymphocytes, platelets, mast cells, and basophils. Immunoglobulin M is the first immunoglobulin produced by fetuses. Immunoglobulin A is the major class of immunoglobulin found in secretions such as tears, saliva, and mucus. This makes it very important in local or mucosal immunity. Immunoglobulin E is the least common serum immunoglobulin and is involved in allergic reactions. The function of IgD is currently unknown and, like IgE, is found in very low concentrations in serum immunoglobulin. (Mayer, n.d.) Immunoglobulin G will be the focus of this research project because of its importance in prenatal health; specifically in newborn foals.

At parturition, foals are considered immunologically naïve and must obtain antibodies from mares' colostrums as there is no placental transfer of immunity in the horse (McCue, 2009). Failure to either obtain colostrum or successfully absorb antibodies is called failure of passive transfer (FPT) and occurs in 10-20% of foals. Causes of FPT include poor quality of the colostrum, dripping milk pre-parturition, and inability to nurse. Obtaining colostrum preferably in the first 6-8 hours post-parturition is essential as the foal's gastrointestinal tract's ability to absorb antibodies declines beyond this time frame and stops after 24 hours post-parturition (McCue, 2009). To determine the success of colostrum absorption, blood samples from the foal can be analyzed. Single Radial Immunodiffusion (SRID) is a common method of determining immunoglobulin quantities and functions by measuring the diameter of a ring formed by the sample when added to an agarose gel. Immunoglobulin concentrations are determined by graphing standards of known concentration. However the procedure requires an incubation period of 18-24 h before results can be analyzed (VMRD; Pullman, WA). The incubation period

of SRID means that results may not be ready until after the loss of absorption of the foal's GI tract which limits opportunities to correct FPT.

Previous studies have been conducted to develop faster methods of determining immunoglobulin concentrations. One example is a foal study on the accuracy of SNAP IgG tests which require only a seven minute incubation period (Pusterla et al., 2002). The SNAP tests showed a total accuracy of 64% when compared to SRID used as a control. Because the SNAP test's results are interpreted through a visual color change, specific counts of IgG cannot be determined, only ranges of <400 mg/dL, 400-800 mg/dL, and >800 mg/dL are given (Pusterla et al., 2002). Another study in calves compared Enzyme Linked Immunosorbant Assays (ELISA) to SRID for determining immunoglobulin concentrations. In the study ELISA showed a 94% agreement with results obtained from SRID. ELISA was found to be more time efficient by requiring only 4 hours of incubation. A noted negative aspect of ELISA is the complex procedure requiring multiple reagents and incubations (Lee et al., 2008). While ELISA has been used to study Immunoglobulin G concentrations in foals, the results have not been correlated with SRID (Erhard et al., 2001).

Due to the high agreement between SRID and ELISA derived Immunoglobulin G concentrations in calves and the time efficiency of the procedure, ELISA may be useful in detecting IgG concentrations in foals to diagnose Failure of Passive Transfer and Partial Failure of Passive Transfer. The purpose of this study is to compare the results of Immunoglobulin G concentrations derived through Enzyme Linked Immunosorbant Assay with a previous study in which concentrations were obtained using Single Radial Immunodiffusion.

MATERIALS AND METHODS

To compare Enzyme-Linked Immunosorbant Assays versus Single Radial Immunodiffusion Assays for determining IgG concentrations, the sera of the eight Quarter Horse mares and their foals born their foals born at The Ohio State University Equine Center from March to June 2008 were evaluated. Foaling data and history were recorded and is shown in Table 1. Mare age ranged from 6 to 18 years old with an average of 10.6 years old. The number of previous pregnancies for each mare ranged from 0 to 9 with an average of 2.4 pregnancies. The length of gestation ranged from 323 days to 359 days with an average of 342.9 days. Five foals were born in April and three foals were born in May. Fifty percent of these foals were male and fifty percent were female.

Each foaling was attended to insure that the initial blood samples were collected prior to the foal nursing. Blood samples were collected via jugular venipuncture on d 0 (immediately after foaling) and 0.5, 1, 7, 14, 21, 28, 56, 84 and 112 d post-partum. These collections followed an approved animal care and use protocol. Blood samples were centrifuged at 1,200 x g for 10 minutes and the serum was decanted. Serum samples were stored at -80°C until assayed. To measure the IgG concentrations separately in the blood serum of the mares and foals commercial Enzyme-Linked Immunosorbant Assay (ELISA) kits (Bethyl Laboratories; Montgomery, TX) were used to determine concentrations of IgGa, IgGT, and IgGb and statistical analysis was performed using 4 parameter curves to compare ELISA derived results with SRID derived results.

ELISAs were performed in a 96-well plate and samples and standards were assayed in duplicate. ELISA functions by binding a purified anti-Horse antibody to the well, then binding the immunoglobulins present in the diluted horse serum to the anti-horse antibody. A third antibody containing a peroxidase and TMB substrate is added to detect the presence of the

immunoglobulins. When the TMB substrate incubation is stopped with a low molar acid solution, a visible color change occurs from blue to yellow. Four-parameter curves were established by assaying standards of known concentration with each plate. Equine IgG has 3 main subclasses: IgGa; IgGb; and IgGT (Lewis et al., 2008). A specific ELISA must be performed to measure the concentration of each subclass. After conducting assays of each IgG subclass, the concentrations were added together to calculate total IgG concentration in mg/dL. Single Radial Immunodiffusion derived IgG concentrations were obtained from a previous study (Kenzig, 2009).

RESULTS

Correlations were only performed with samples present in both datasets. No IgG was detected in foals at 0 d, before nursing, in the SRID study (Kenzig, 2009). In the present study, IgG was detected in foals with an average concentration of 60.51 mg/dL at 0 d (Table 2). The mare and foal datasets were correlated separately and in mares no correlation ($r = -0.06$) was found, while in foals a weak correlation ($r = 0.30$) was found. The correlation for the entire dataset is weak ($r = 0.17$). In foals, the strongest correlation ($r = 0.74$) was found at Day 56 and the strongest negative correlation ($r = -0.28$) was found at 112 d (Figure 2.). The strongest correlation for mares ($r = 0.57$) was found at 1 d and the strongest negative correlation in mares ($r = -0.54$) was found at 56 d (Figure 1.).

There were several discrepancies between ELISA and SRID for values measured in the first 24 hours post-foaling which is the window of absorption for colostrums in foals. At 12h, post-nursing, Foals 2 and 5 had SRID immunoglobulin concentrations of 400 mg/dL and 580 mg/dL, respectively, while the ELISA immunoglobulin concentrations of Foals 2 and 5 were 1304.09 mg/dL and 1837.62, respectively. In addition, at 12h, Foals 4 and 8 had SRID

immunoglobulin concentrations of 1150 mg/dL and 1600 mg/dL, respectively, while the ELISA immunoglobulin concentrations of the Foals 4 and 8 were 780.01 mg/dL and 532.19 mg/dL, respectively. Correlations by day for mares and foals are listed in Table 2.

DISCUSSION

The results of the study may present a conflict with research comparing ELISA with SRID values. In a previous study in calves, an agreement of 94% was found between ELISA and SRID immunoglobulin G concentrations (Lee et al., 2008). In the present study, correlation was used for analysis and showed only a weak correlation in foals and no correlation in mares. At 12 h, after receiving colostrum, results differed greatly in four of the foals. Using SRID, Foals 2 and 5 would be diagnosed with Partial Failure of Passive Transfer as their IgG concentrations were 400 mg/dL and 580 mg/dL, respectively. While using ELISA, their IgG concentrations were both calculated to be above the 800 mg/dL threshold needed for transfer of immunity. A similar outcome occurred with Foals 4 and 8 in which the ELISA derived concentrations were between 400 and 800 mg/dL, indicating PFPT, while the SRID values were above 800 mg/dL. A potential for a false positive or false negative diagnosis of FPT or PFPT exists for foals whose IgG concentrations were derived solely from ELISA. Two possibilities as to why ELISA displayed such low correlation with SRID are the effects of long term storage at -80°C, as the samples are from 2008, and the complexity of ELISA, which makes the assay prone to human error. Repeated freeze and thaw cycles would be expected to degrade the immunoglobulins resulting in lower IgG concentrations being detected. ELISA human error is unlikely as samples were assayed in duplicate and samples were reassayed if the covariance was above 10%. Neither human error nor long term freezing can account for the difference in correlation between mares and foals. Further research is needed to determine why the results of

this study conflict with a similar study in calves and to analyze the factors contributing to the variation in ELISA derived and SRID derived IgG concentrations.

TABLES AND FIGURES

Figure 1.

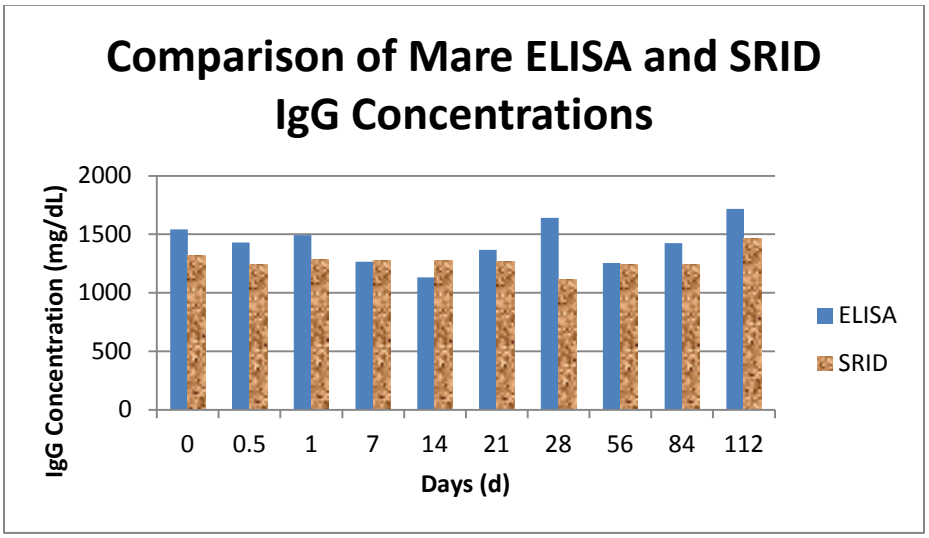

Figure 2.

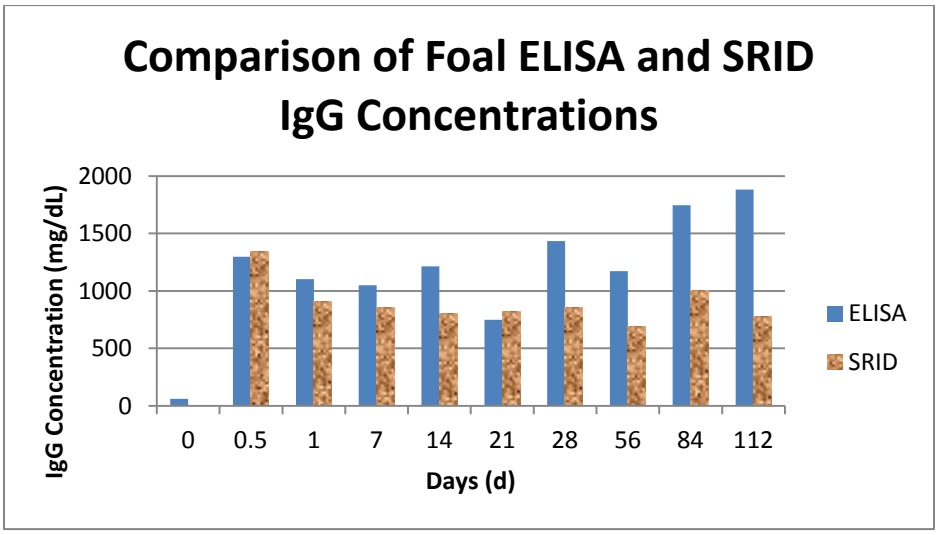

Table 1.

Mare ID	Mare Age (yrs)	Previous Pregnancies	Days Gestation	Month Foaled	Foal Sex
M1	6	0	347	April	Male
M2	9	4	344	April	Female
M3	15	1	343	April	Male
M4	11	1	359	April	Male
M5	7	2	349	April	Female
M6	8	1	323	May	Female
M7	11	1	345	May	Male
M8	18	9	333	May	Female

Table 2.

IgG Concentration (mg/dL) in Mares and Foals										
Source	0d	0.5d	1d	7d	14d	21d	28d	56d	84d	112d
Mare Serum										
(n=)	6	6	6	8	7	8	8	7	8	6
Correlation (r)	-0.019	-0.50	0.57	0.10	0.12	-0.36	0.22	-0.54	-0.43	-0.50
Total Mare Correlation (r)	-0.07									
ELISA Mean +/- SD	1542.02 +/- 391.94	1428.81 +/- 411.68	1493.44 +/- 242.77	1266.58 +/- 331.59	1132.15 +/- 186.79	1366.42 +/- 254.89	1640.62 +/- 687.32	1253.72 +/- 214.14	1424.17 +/- 287.02	1718.83 +/- 391.32
ELISA Range	1079.9-2116.01	1200.5-2241.53	1251.3-1915.11	853.61-1883.41	951.57-1466.68	1095.03-1899.00	1075.68-2995.52	980.88-1558.71	1147.10-1966.72	1209.92-2185.53
SRID Mean +/- SD	1316.67 +/- 335.66	1241.67 +/- 308.90	1283.33 +/- 537.28	1278.75 +/- 634.36	1273.57 +/- 744.74	1268.75 +/- 670.85	1110 +/- 614.77	1242.86 +/- 363.35	1236.88 +/- 682.35	1466.67 +/- 733.94
SRID Range	800-1600	800-1600	800-2200	580-2200	400-2200	400-2200	400-2200	800-1600	565-2200	400-2200
Foal Serum										
(n=)	7	5	7	7	8	7	7	8	7	7
Correlation	0	0.26	0.65	0.07	0	0.60	0.09	0.74	0.34	-0.28
Total Foal Correlation	0.30									
ELISA Mean +/- SD	60.51 +/- 128.92	1297.611 +/- 649.60	1102.08 +/- 425.79	1050.21 +/- 567.47	1213.54 +/- 592.20	748.10 +/- 338.54	1433.44 +/- 1221.06	1171.067 +/- 180.95	1744.63 +/- 623.78	1882.68 +/- 428.37
ELISA Range	5.16 - 351.84	532.19 - 2034.14	614.74 - 1683.62	376.92 - 1875.34	280.67 - 1923.04	242.52 - 1183.36	351.74 - 3329.06	920.98 - 1409.41	1293.42 - 2980.46	1345.45 - 2479.50
SRID Mean +/- SD	0 +/- 0	1346 +/- 1039.27	904.29 +/- 494.26	856.43 +/- 421.47	805.63 +/- 352.41	823.57 +/- 549.44	853.57 +/- 657.83	686.25 +/- 323.10	1001.43 +/- 637.35	772.86 +/- 360.36
SRID Range	0	400-3000	200-1600	280-1600	400-1600	400-1600	280-2200	280-1150	280-1600	400-1150
Total Correlation	0.17									

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